PAPER

Intrathecal inflammation precedes development of Alzheimer's disease

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Objectives: To analyse the cerebrospinal fluid (CSF) values of the proinflammatory cytokines, interleukin 1β (IL1β), tumour necrosis factor α (TNFα), GM-CSF, of the anti-inflammatory cytokine TGFβ, of tau protein, a marker for neurodegeneration, and of β amyloid (Aβ), a protein involved in the formation of senile plaques, in prospectively followed up patients with mild cognitive impairment (MCI).
Methods: Analyses of CSF levels of TNFα, IL1β, GM-CSF, TGFβ, βa, and tau protein were performed using ELISA in 56 patients with MCI who were followed up prospectively and in 25 age matched, healthy controls.
Results: Patients with MCI displayed significantly higher levels of TNFα and tau protein and significantly lower levels of TGFβ and Aβ compared with the healthy controls. After nine months of follow up, 25 patients still displayed MCI while the remaining 31 patients had progressed to Alzheimer's disease (AD). Only MCI patients who progressed to AD at follow up, showed significantly higher CSF levels of TNFα than controls. In addition, reduced CSF-Aβ42 levels were only found in MCI patients that progressed to AD, further supporting the notion that disturbed metabolism of Aβ is an early finding in AD.
Conclusions: These results demonstrate increased production of the proinflammatory cytokine, TNFα and decreased production of the anti-inflammatory cytokine TGFβ in patients with MCI at risk to develop AD, suggesting a propensity towards inflammation in this patient group and indicating that CNS inflammation is a early hallmark in the pathogenesis of AD.

Alzheimer's disease (AD) is characterised by atrophy of the brain subsequent to neuronal and synaptic degeneration, decreased dendritic arborisation, as well as formation of neurofibrillary tangles and senile plaques (SP). A protein of 40–42 amino acid length, with a high tendency to aggregate, called β amyloid protein (Aβ), has been identified as the major component of the SP.[1] Aβ is derived from a 90–140 kDa precursor, called amyloid precursor protein (APP).[2]

The cause of AD and reasons for its progressive course are still unknown but involvement of the immune system in the pathogenesis has been discussed for years. For example, $CD4^+$ and $CD8^+$ T lymphocytes are present in affected AD brain tissue.[3] In addition, reactive microglial cells have been demonstrated to express class-II molecules of the major histocompatibility complex (MHC) and Fc receptors.[4] Thus, all the appropriate elements necessary for an immune response are present locally in brains of AD patients.

Both activated microglia and T lymphocytes can be a source of cytokine production.[5] Indeed, interleukin (IL) 1, IL6, and tumour necrosis factor (TNF) α are detected in brains of AD patients.[6–8] These cytokines may be involved in the pathogenesis of AD, for example, by promoting local inflammatory responses.[9] IL1 has also been demonstrated to increase the synthesis of APP mRNA in human endothelial cells,[10] suggesting a direct role for this cytokine in the formation of SP. Interestingly, the IL1 receptor antagonist (IL1ra) naturally occurring in the brain,[11] and blocking interaction of IL1 with its receptor,[12] displays neuroprotective properties with respect to ischaemic and excitotoxic brain damage in experimental rat models.[13] Furthermore, TNFα, a powerful cytokine inducing apoptosis in the extraneural compartments of the body, has been demonstrated to protect rat hippocampal, septal and cortical cells against metabolic-excitotoxic insults[14] and to facilitate regeneration of injured axons.[15] More importantly, TNFα and TNFβ protect neurons against Aβ triggered toxicity.[16]

We have previously demonstrated increased levels of the proinflammatory cytokines, TNFα, GM-CSF and of the anti-inflammatory cytokine TGFβ levels in the cerebrospinal fluid (CSF) of patients with established AD.[17–20] Thus, a body of evidence suggests an increased cytokine production in the brain of AD patients and the potential involvement of these cytokines in the pathophysiology of AD. However, it is not known whether the cytokine production is a late event in AD, if it is an early step in the pathogenesis of AD, or if it even precedes the diagnosis of AD. If the last alternative would occur, the idea of inflammatory component in the pathophysiology of AD would be further strengthened.

The aim of this study was to analyse (1) the CSF levels of the proinflammatory cytokines, IL1β, TNFα, GM-CSF, and of the anti-inflammatory cytokine TGFβ in patients with mild cognitive deficit (MCI) known to proceed with time to full blown AD.

METHODS

Patients

Fifty six patients with MCI, 28 men and 28 women, 51–90 years of age (mean (SEM) 74 (1) years), all patients at the Neuropsychiatric Department, Piteå River Valley Hospital, Piteå, Sweden, were consecutively incorporated into the study during a four year period. The patients underwent a thorough clinical examination including medical history, physical, neurological and psychiatric status, psychological tests, laboratory screening tests, ECG, chest radiographs, EEG, and CT scans of the brain. All patients underwent a comprehensive

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; IL, interleukin; TNF, tumour necrosis factor; IL1ra, IL1 receptor agonist; Aβ, β amyloid protein; MMSE, mini-mental state examination; MCI, mild cognitive impairment; ELISA, enzyme linked immunosorbent assay; SP, senile plaque; APP, amyloid precursor protein; VEGF, vascular endothelial growth factor

neuropsychological assessment, and a detailed evaluation of the instrumental ADL status including non-memory symptoms of dementia by an occupational therapist, as described previously in detail.[21–23] We also interviewed the closest relatives for history of cognitive, occupational, and social impairment. The clinical symptomatology was evaluated, and the following symptoms recorded as absent or present: memory disturbances, dysphasia, dyspraxia, visuospatial disturbances, and personality change. The mini-mental state examination (MMSE) was used to evaluate the degree of intellectual impairment.[24] The initial MMSE score was (mean (SEM)) 28.6 (0.2) in all the patients.

All the patients were followed up prospectively and examined about nine months later (range 2–36 months). Twenty five patients still displayed a mild memory disturbances only, while the remaining 31 patients had developed additional cognitive symptoms, either evident from the medical history or the clinical examination, and had thus progressed to AD with dementia. The MMSE, which was only used for grading of cognitive symptoms, and not for evaluation of conversion to AD, was significantly lower in the group that had progressed to AD (27.1 (0.4), range 22–30) than in the group that did not progress (28.6 (0.3), range 25–30).

Mild cognitive impairment was diagnosed in patients with no other cognitive symptoms than memory impairment, following the guidelines by Peterson and colleagues.[25] Memory impairment was verified by neuropsychological testing. No patient had impairment of activities of the daily living, or fulfilled the DMS-IV criteria[26] for dementia. An inclusion criterion was also a MMSE score at baseline of 27 or above.

AD was defined as dementia with predominant instrumental deficits, as well as loss of memory, but without clinical evidence of frontal lobe dementia, dementia of Lewy body type, or cerebrovascular changes such as white matter lesions.[27] All patients that had progressed at follow up fulfilled the NINCDS-ADRDA criteria for probable AD.[28] AD had an early onset (before the age of 65 years) in nine patients and a late onset (after 65 years) in 22 patients.

For control purposes, CSF samples from 25 healthy controls, 50–83 years of age (mean (SEM) 68 (1)), without any neurological diseases or deficit, were obtained to establish normal levels of cytokines.

No patients or controls displayed symptoms or signs (for example, fever) of inflammatory or infectious conditions. In addition, all of them had erythrocyte sedimentation rate (ESR) within reference values. Patients with malignant diseases were excluded from all the study groups.

In both the MCI and the control group, CSF samples were obtained by lumbar puncture in the L3/L4 or L4/L5 interspace at the admission of the patients to the study. The first 12 ml of CSF was collected in plastic (polypropylene) tubes, to avoid possible absorbance of proteins to the tube walls.[21] All CSF samples were gently mixed to avoid possible gradient effects. No CSF sample contained more than 500 erythrocytes per µl. The CSF samples were centrifuged at 2000×*g* for 10 minutes to eliminate cells and other insoluble material and were then immediately frozen, and stored at −80°C pending biochemical analyses, without being thawed and re-frozen. The CSF and serum samples of the patients were obtained for routine analyses, protein electrophoresis, cytology, and analyses of TNFα, IL1β, GM-CSF, TGFβ, Aβ, and tau protein.

This study was approved by the ethics committees of the Universities of Göteborg and Umeå

Reagents and procedures

Cytokine analysis

Levels of GM-CSF in CSF samples were quantified by an enzyme linked immunosorbent assay (ELISA) using GM-CSF specific monoclonal unlabelled antibodies (Pharmingen, San Diego, CA) for coating (2 µg/ml) of polystyrene flat bottom plates (4°C, overnight), followed by washing with TRIS 0.05% Tween-20 and blocking with 1% BSA in TRIS-NaCl for one hour at 37°C. After emptying, the plates were incubated for two hours at 37°C with CSF samples diluted 1:5 in TRIS-NaCl containing 1% BSA. Biotinylated GM-CSF specific monoclonal antibodies (Pharmingen) were added and incubated overnight at 4°C. The plates were washed again and incubated with ExtrAvidin alkaline phosphatase (Sigma, St Louis, MO). The enzyme substrate was then added, and optical density was determined in a Titertek Multiscan Photometer (Flow Laboratories, McLean, VA) at 405 nm. The concentration of GM-CSF was calculated using a standard curve based on known quantities of human recombinant GM-CSF (Quantikine R&D Systems, Minneapolis, MD).

Levels of IL1β, TNFα, and TGFβ in cerebrospinal fluid samples were estimated by ELISA (Quantikine R&D Systems, Minneapolis, MD).

The detection level for TNFα, IL1β, GM-CSF, and TGFβ were 0.2 pg/ml, 0.1 pg/ml, 0.04 ng/ml, and 7 pg/ml , respectively. All values below the detection levels were considered to be negative.

Analysis of tau protein

Tau protein levels in CSF were determined using a sandwich ELISA, constructed to measure the level of total tau, containing both phosphorylated and non-phosphorylated protein, as described previously in detail.[29]

Analysis of β amyloid protein

CSF-Aβ42 was determined using a sandwich ELISA (INNOTEST β amyloid(1–42), Innogenetics, Ghent, Belgium) constructed to specifically measure β amyloid1–42, as previously described.[26 30]

Statistics

Statistical analysis regarding the differences between means was carried out by the Mann-Whitney U test. Paired data were analyded with Wilcoxon's signed rank test. A χ^2 test was used to analyse categorical data. Spearman's rank order correlation method was used to calculate correlations and the level of significance of the correlations.

In addition to the non-parametric approach, the data concerning the CSF levels of TNFα, TGFβ, IL1β, tau, and Aβ were transformed to normalise the distribution and thereby permit use of parametric techniques. For the logaritmic transformation, all values for TNFα and IL1β below the detection levels were considered to be at the detection level. A two way analysis of variance with sex and age as factors and CSF-TNFα, CSF-TGFβ, CSF-tau; CSF-Aβ as effect variables was performed. Sex and age did not contribute to the variance in any of the tests performed and could be excluded from further analyses. Unpaired Student's *t* test was then used to compare the different groups as shown in the table 3. A p value <0.05 was considered statistically significant.

To assess the propensity to inflammatory reaction in the CNS, the ratio between TNFα and TGFβ level was calculated in each patient as follows: CSF levels of TNFα (pg/ml) divided by CSF levels of TGFβ (pg/ml), multiplied by 1000. The values below detection levels were set to 0.01.

RESULTS

Patients

Table 1 shows the clinical features of the patients. The patients who progressed to AD at the follow up nine months later were slightly but significantly ($p=0.03$) older than patients who did not. As expected, the patients who progressed to AD displayed significantly higher ($p=0.007$) degree of cognitive impairment at the follow up than patients who did not. Also, patients with AD at the follow up showed a significant decrease

Table 1 Clinical features of MCI patients included in the study

Clinical features	All patients (n=56)	Patients with AD at follow up (n=31)	Patients with MCI at follow up (n=25)
Age	72 (1)	74 (1)	69 (2)
Initial MMSE	28.9 (0.1)	28.8 (0.2)	29.0 (0.2)
MMSE at follow up	27.8 (0.2)	27.1 (0.4)***	28.6 (0.3)
MMSE change	−1.1 (0.3)	−1.7 (0.4)	−0.4 (0.3)

***p<0.001 in comparison with initial MMSE.

Table 2 CSF levels of TNFα, TGFβ, IL1β, GM-CSF, tau, and Aβ in prospectively followed up MCI patients

	All patients	Patients with AD at follow up	Patients with MCI at follow up	Healthy controls
	(n=56)	(n=31)	(n=25)	(n=25)
TNFα	0.4 (0.1)***	0.4 (0.1)***	0.4 (0.1)**	0.2 (0.1)
	0.3 (0.5)	0.3 (0.1)	0.3 (0.4)	0.0 (0.0)
TGFβ	124 (20)***	104 (6)***	149 (43)***	159 (10)
	99 (53)	99 (46)	94 (63)	150 (61)
IL1β	0.04 (0.02)	0.04 (0.02)	0.05 (0.04)	0.0 (0.0)
	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
GM-CSF	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	18.0 (9.0)
	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (40.0)
Tau	600 (29)***	628 (29)***	564 (53)**	314 (36)
	568 (313)	588 (281)	476 (382)	299 (196)
Aβ	683 (42)**	602 (43)***	784 (72)	931 (55)
	557 (410)	555 (167)	698 (600)	940 (219)

All data are presented as mean (SEM) (first row for each parameter) and median (interquartile range) (second row for each parameter) and are expressed in pg/ml for TNFα, ILβ, TGFβ, and Aβ, μ/ml for tau, and in ng/ml for GM-CSF. ***p<0.001 in comparison with controls. **p<0.01 in comparison with controls.

(p=0.0004) of cognitive impairment compared with their initial degree of impairment, whereas the remaining subjects did not show a similar trend.

Intrathecal TNFα levels

Forty one of 56 patients but only 7 of 33 controls had detectable levels of TNFα in CSF (p<0.0001). As table 2 shows, the levels of TNFα in the CSF were significantly increased (p=0.0009) in patients with MCI (mean (SEM) 0.4 (0.1) pg/ml) compared with the controls (0.2 (0.1) pg/ml). After logaritmic transformation of the data, the differences between the groups remained significant (table 3). In addition, when the TNFα levels of the patients who developed AD at the follow up and of the patients who did not were analysed separately, only MCI patients who progressed to AD at follow up, showed significantly higher CSF levels of TNFα compared with controls (table 3). The TNFα levels in CSF did not correlate to any of the clinical features including age of the patients (r=−0.05, NS), initial MMSE score (r=0.04, NS), or MMSE score at the follow up (r=0.03, NS).

Intrathecal IL1β and GM-CSF levels

Six of 56 patients but none of 33 controls had detectable levels of IL1β (p<0.05). The CSF levels of IL1β did not differ significantly between patients and controls (table 2 and table 3). However, the IL1β levels were significantly correlated both to MMSE at the first examination (r=0.46; p=0.0006) and at the follow up (r=0.34; p=0.001) as well as to the change in MMSE between the two examinations (r=0.30; p=0.03) and to the age of the patients (r=0.32; p=0.02).

None of the patients and four of the controls displayed detectable GM-CSF levels in CSF (NS). The CSF levels of GM-CSF did not differ significantly between patients and controls (table 2).

Intrathecal TGFβ levels

All the patients and all the controls displayed detectable levels of TGFβ in the CSF (NS). The levels of TGFβ in the CSF were significantly decreased (p=0.0001) in patients with MCI (mean (SEM) 124 (20) pg/ml) compared with the controls (159 (10) pg/ml) (table 2 and table 3). When the TGFβ levels of the patients who developed AD at the follow up and of the patients who did not were analysed separately, both groups displayed significantly decreased and similar TGFβ levels compared with the controls (table 2 and table 3). To assess the propensity to inflammation in the people studied, the ratio between TNFα and TGFβ was calculated as described in

Table 3 Significance levels between the patient groups and controls as well as between patients who converted to AD at the follow up and patients who did not

	Significance levels				
	TNFα	TGFβ	IL1β	Aβ42	Tau
All patients *v* controls	0.046	0.002	NS	0.003	0.0001
AD *v* MCI at follow up	NS	NS	NS	0.042	NS
MCI at follow up *v* controls	NS	0.05	NS	NS	0.003
AD at follow up *v* controls	0.039	0.0004	NS	0.0001	0.0001

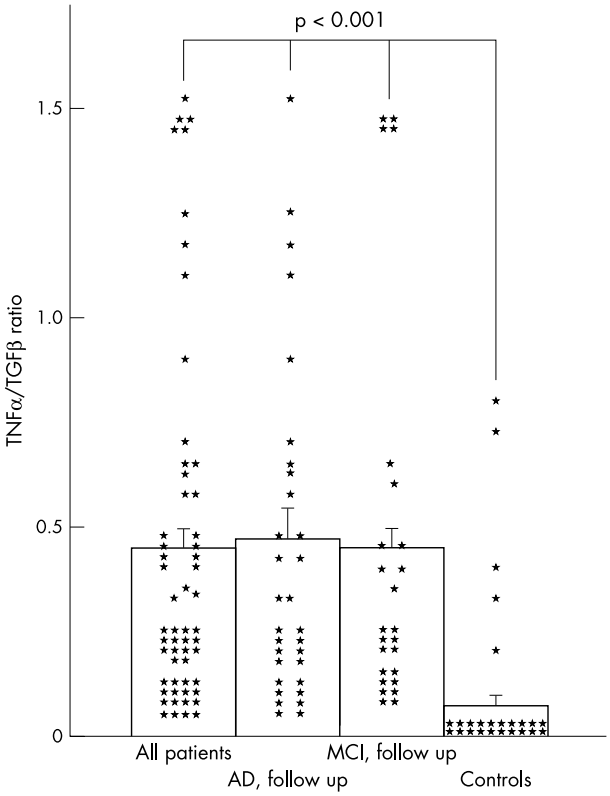

Figure 1 Ratio between CSF levels of TNFα and TGFβ in 56 patients with MCI, 31 patients who develop AD at the follow up, 25 patients with remaining MCI at the follow up, and in 27 healthy controls (mean (SEM)). The ratio was calculated as described in Methods. High ratio indicates increased propensity to inflammation because of increased TNFα levels and decreased TGFβ levels as compared with the healthy controls. ***p<0.0001.

Methods. All the patient groups studied displayed significantly higher TNFα to TGFβ ratios than healthy controls (fig 1), indicating a significantly higher proinflammatory state in the patients at risk to develop AD.

The TGFβ levels in CSF did not correlate to any of the clinical features including age of the patients (r=0.2; NS), initial degree of intellectual impairment (r=–0.03, NS) or degree of intellectual impairment at the follow up (r=–0.03, NS).

Intrathecal tau levels

All the patients and all the controls displayed detectable levels of tau in the CSF (NS). The CSF levels of tau were significantly higher (p=0.009) in patients with MCI, and both in the group who developed AD at the follow up (p=0.0001) and in patients with remaining MCI (p=0.01), compared with the healthy controls (table 2 and table 3). The intrathecal tau levels did not differ significantly between patients with AD and patients with MCI at the follow up (table 3). The intrathecal levels of tau were not correlated to the age of the patients (r=–0.16, NS). In addition, the intrathecal levels of tau protein were not correlated either to the initial degree of intellectual impairment (r=–0.014, NS), or to the degree of intellectual impairment at the follow up (r=–0.23; NS). However, when the patients who progressed to AD and the patients who did not were analysed separately, the levels of tau were significantly correlated to the MMSE levels at the follow up only in patients with AD (r=–0.41; p=0.02), but not in patients with remaining MCI (r=0.023; NS).

The levels of tau were correlated (r=0.42; p=0.002) to the levels of IL1β in all patients with MCI. Interestingly, when analysed separately, the levels of tau were significantly correlated to the levels of IL1β, only in patients who developed AD at the follow up (r=0.45; p=0.01) but not in patients with remaining MCI (r=0.37; NS). The levels of tau were not significantly correlated to the levels of the other cytokines in any of the groups studied.

Intrathecal levels of Aβ42

All the patients and all the controls displayed detectable levels of Aβ42 in the CSF (NS). The CSF levels of Aβ42 were significantly lower (p=0.004) in all patients with MCI, compared with the healthy controls (table 2 and table 3). Subsequent analysis revealed that only patients who developed AD at the follow up displayed significantly lower Aβ42 than controls whereas patients with remaining MCI did not (table 2 and table 3). The intrathecal levels of Aβ42 were not correlated to the age of the patients (r=–0.03, NS). In addition, the intrathecal levels of Aβ42 were not correlated either to the initial degree of intellectual impairment (r=0.007, NS), or to the degree of intellectual impairment at the follow up (r=0.18; NS).

The levels of Aβ42 were not correlated (r=0.24; NS) to the levels of IL1β in all patients with MCI. However, upon subsequent analysis, the levels of Aβ42 were significantly correlated to the levels of IL1β, only in patients who developed AD at the follow up (r=0.37; p=0.04) but not in patients with remaining MCI (r=0.09; NS). The levels of Aβ42 were not significantly correlated to the levels of the other cytokines in any of the groups studied.

DISCUSSION

Our study has shown increased levels of the proinflammatory cytokine TNFα and decreased levels of the anti-inflammatory cytokine TGFβ in the CSF of patients with MCI compared with healthy controls. Patients with MCI also displayed higher levels of tau, a marker for neurodegeneration, and lower levels of Aβ42, a protein involved in the formation of senile plaque in AD brain. In addition, the levels of IL1β, a proinflammatory cytokine involved in neurotoxicity and in the production of APP, the precursor molecule to Aβ42, were significantly correlated to the levels of tau and of Aβ in patients with MCI who progressed to full blown AD at the follow up nine months later.

An increasing body of evidence suggests that inflammation in CNS, mediated by glial activation and production of inflammatory mediators such as cytokines and complement, plays a part for pathophysiology of AD.[31] In this respect, we have previously demonstrated intrathecal production of TNFα in patients with AD.[17 18] This study suggests that the intrathecal production of TNFα may be not only viewed as an early hallmark of AD but may actually precede clinically manifest AD. This finding is important because it indicates that cytokine release is not a late consequence of the disease but rather might be one of the initiating factors.

In addition, when the normalised TNFα levels of the patients who developed AD at the follow up and of the patients who did not were analysed separately, only MCI patients who progressed to AD at follow up, showed significantly higher CSF levels of TNFα than the controls, suggesting an involvement of this cytokine in the progression of dementia. However, we did not find any significant differences regarding the initial TNFα mean levels between patients who proceeded to overt AD and patients who did not at the follow up, nine months later. One possible explanation is that the observation period is too short and that most patients would convert to AD later on. The lack of significant difference between the groups of patients with MCI who progressed to AD and those who do not may also be attributable to the small sample size of the subgroups. Another possibility is that a second event is required for development of AD in MCI patients. Such an event could be additional production of cytokines regulating amyloidogenesis. Interestingly, we have previously

demonstrated that patients with overt, established AD displayed high intrathecal levels of TGFβ[20] whereas, in the present study, that patients with MCI displayed significantly decreased levels of this cytokine. Combined, these data suggest that the production of TGFβ is one of the factors that differ between patients with MCI and with overt AD. In the cytokine network, TGFβ acts as an anti-inflammatory cytokine, inhibiting the production of the proinflammatory cytokines such as TNFα, IL1, and IL6 by astrocytes,[32 33] and suppressing the activation and proliferation of microglia.[33] The production of TGFβ is regulated by other cytokines including TNFα.[34] In patients with MCI, the increased levels of TNFα may trigger the production of TGFβ by a negative feed back mechanism to counteract the proinflammatory effects of TNFα. However, TGFβ has other properties of interest in the case of AD, for example, it is known to promote amyloidogenesis in experimental models of AD indicating that it may be a risk factor for developing AD.[35] In addition, TGFβ is also implicated in angiogenesis. This cytokine has been demonstrated to induce the production of vascular endothelial growth factor (VEGF),[36 37] one of the most potent angiogenic growth factors. TGFβ has also been demonstrated to be activated during hypoxia[38 39] and it has been speculated that hypoxia triggered TGFβ activation may be a prerequisite for the upregulation of VEGF and stimulation of angiogenesis.[39] Interestingly, the nun study has shown that patients with both vascular pathology and AD pathology displayed severe dementia.[40] Thus, we speculate that, in patients with MCI who convert to AD, a shift occurs in the production of TGFβ, from inhibition to overproduction and that this event could be the second prerequisite to trigger AD pathology. One of the limitations of this study was that the follow up period was comparativly short and analyses of cytokines in CSF were not performed at the follow up examination. Further prospective studies are needed to evaluate the kinetics of cytokine production in patients with mild cognitive deficit at risk to develop dementia.

Increased levels of CSF-tau, reflecting the neuronal degeneration have previously been found in patients with AD.[29 41 42] Similarly, decreased levels of CSF-Aβ42, the main component of SP, has been found in patients with AD,[21 42] possibly reflecting an increased consumption of this protein caused by the formation of SP. This study suggests that increased neuronal degeneration is evident also in patients with mild cognitive deficit risking to develop AD and that increased levels of tau protein in CSF is an early marker of AD. In addition, the decrease of Aβ42 was more prominent in MCI patients developing AD than in MCI patients who did not, suggesting that Aβ42 is early involved in the pathophysiology of AD. Interestingly, the levels of these proteins were correlated to the levels of IL1β in patients developing AD in the follow up but not in the remaining patients. In this respect, it should be noted that immunoreactivity for IL1β has been detected in AD brains.[7] IL1β has also been demonstrated to increase the synthesis of APP mRNA in human endothelial cells,[10] suggesting a direct role for this cytokine in the formation of SP. In addition, IL1β has been demonstrated to induce neuronal degeneration in animal models[43] and to mediate Aβ induced inflammatory reaction.[44] Thus, the correlation between tau, a marker for neurodegeneration, Aβ, involved in the formation of SP and IL1β in patients with MCD who are soon to develop AD suggests that IL1β is an early initiator/mediator of this condition.

In conclusion, our results demonstrate increased production of the proinflammatory cytokine, TNFα and decreased production of the anti-inflammatory cytokine TGFβ in patients with MCI at risk to develop AD, as well as correlation between IL1β and markers for neurodegeneration and amyloidogenesis, suggesting a propensity towards inflammation in these patients and indicating that CNS inflammation precedes development of AD.

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REFERENCES

1 **Masters CL**, Simms G, Weinman NA, *et al*. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci* 1985;**82**:4245–9.
2 **Banati RB**, Beyreuter K. Alzheimer's disease. In: Kettenman H, Ransom BR, eds. *Neuroglia*. New York: Oxford University Press, 1995:1027–43.
3 **McGeer PL**, Akiyama H, Itagaki S, *et al*. Immune system response in Alzheimer's disease. *Can J Neurol Sci* 1989;**16**:516–27.
4 **McGeer PL**, Itagaki S, Tago H, *et al*. Reactive microglia in patients with senile dementia of Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neurosci Lett* 1987;**79**:195–200.
5 **St Pierre BA**, Merill JE, Dopp JM. Effects of cytokines on the CNS cells: glia. In: Ransohoff RM, Benveniste EN, eds. *Cytokines and the CNS*. Boca Raton, FL: CRC Press, 1996:151–68 .
6 **Bauer J**, Ganter U, Strauss S, *et al*. The participation of interleukin-6 in the pathogenesis of Alzheimer's disease. 45th forum in Immunology. *Res Immunol* 1992;**143**:650–7.
7 **Griffin WST**, Stanley LC, Ling C, *et al*. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci USA* 1989;**86**:7611–15.
8 **Dickson DW**, Lee SC, Mattiace LA, *et al*. Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. *Glia* 1993;**7**:75–83.
9 **Vandenabeele P**, Fiers W. Is amyloidogenesis during Alzheimer's disease due to an IL-1-/IL-6-mediated "acute phase response" in the brain? *Immunol Today* 1991;**12**:217–19.
10 **Goldgaber D**, Harris HW, Hla T, *et al*. Interleukin 1 regulates synthesis of amyloid β-protein precursor mRNA in human endothelial cells. *Proc Natl Acad Sci USA* 1989;**86**:7606–10.
11 **Licinio J**, Wong M, Gold PW. Localization of interleukin-1 receptor antagonist mRNA in rat brain. *Endocrinology* 1991;**129**:562–4.
12 **Dinarello CA**, Thompson RC. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. *Immunol Today* 1991;**12**:404–10.
13 **Relton JK**, Rothwell NJ. Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. *Brain Res Bull* 1992;**29**:243–6.
14 **Cheng B**, Christakos S, Mattson MP. Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. *Neuron* 1994;**12**:139–53.
15 **Schwartz M**, Solomon A, Lavie V, *et al*. Tumor necrosis factor facilitates regeneration of injured central nervous system axons. *Brain Res* 1991;**545**:334–8.
16 **Barger SW**, Hörster D, Furukawa K, *et al*. Tumor necrosis factors α_and β protect neurons against amyloid β-peptide toxicity: Evidence for involvement of a κB-binding factor and attenuation of peroxide and Ca^{2+} accumulation. *Proc Natl Acad Sci USA* 1995;**92**:9328–32.
17 **Tarkowski E**, Wallin A, Blennow K, *et al*. Local production of TNF-α, a potent neuroprotective agent, in Alzheimer disease and vascular dementia. *J Clin Immunol* 1999;**19**:223–30.
18 **Tarkowski E**, Liljeroth A-M, Nilsson Å, *et al*. TNF gene polymorphism and its relation to intracerebral production of TNF-α and -β in AD. *Neurology* 2000;**54**:2077–81.
19 **Tarkowski E**, Wallin A, Regland B, *et al*. Local and systemic cytokine release in patients with Alzheimer disease and vascular dementia. *Acta Neurol Scan* 2001;**103**:166–74.
20 **Tarkowski E**, Issa R, Sjögren M, *et al*. Increased intrathecal levels of the angiogenic factors VEGF and TGF-β in Alzheimer's disease and vascular dementia. *Neurobiol Aging* 2002;**23**:237–43.
21 **Andreasen N**, Hesse C, Davidsson P, *et al*. Cerebrospinal fluid β-amyloid$_{(1–42)}$ in Alzheimer's disease: differences between early- and late-onset AD and stability during the course of disease. *Arch Neurol* 1999;**56**:673–80.

22 **Andreasen N**, Blennow K, Sjödin C, *et al*. Prevalens and Incidens of clinically diagnosed memory impairments in a geographically defined general population in Sweden. The Piteå Dementia project. *Neuroepidemiology*1999;**18**:144–55.
23 **Andreasen N**, Minthon L, Vanmechelen E, *et al*. Cerebrospinal fluid tau and Aβ42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment. *Neurosci Lett* 1999;**273**:5–8.
24 **Folstein MF**, Folstein SE, Mc Hugh P. "Mini-mental state" A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;**12**:189–98.
25 **Petersen RC**, Smith GE, Ivnik RJ, *et al*. Apolipoprotein E, status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. *JAMA* 1995;**273**:1274–8.
26 **American Psychiatric Association**. *Diagnostic and statistical manual of mental disorders*. 4th edn. Washington, DC: American Psychiatric Association, 1994.
27 **Wallin A**, Blennow K, Scheltens PH. Research criteria for clinical diagnosis of "pure" Alzheimer's disease. *Drugs of Today* 1994;**30**:265–73.
28 **Mc Khann G**, Drachman D, Folstein M, *et al*. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;**34**:939–44.
29 **Blennow K**, Wallin A, Ågren H, *et al*. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer's disease? *Mol Chem Neuropathol* 1995;**26**:231–45.
30 **Vanderstichele H**, Blennow K, D'Heuvaert N, *et al*. Development of a specific diagnostic test for measurement of b-amyloid(1–42) in CSF. In: Fisher A, Hanin I, Yoshida M, eds. *Progress in Alzheimer's and Parkinson's diseases*. New York: Plenum Press, 1998:773–8.
31 **Akiyama H**, Barger S, Barnum S, *et al*. Inflammation and Alzheimer's disease. *Neurobiol Aging* 2000;**21**:383–421.
32 **Benveniste EN**, Tang LP, Law RM. Differential regulation of astrocyte TNF-α expression by the cytokines TGF-β, IL-6 and IL-10. *Int J Dev Neurosci* 1995;**13**:341–9.
33 **Suzumara A**, Sawada M, Yamamoto H, *et al*. Transforming growth factor-β suppresses activation and proliferation of microglia in vitro. *J Immunol* 1993;**151**:2150–8.
34 **Tracey KJ**. Tumor necrosis factor. In: Remick DG, Friedland JS, eds. *Cytokines in health and disease*. 2nd edn. NewYork: Marcel Dekker, 1997:223–39.
35 **Wyss-Coray T**, Masliah E, Mallory M, *et al*. Amyloidogenic role of cytokine TGF-β1 in transgenic mice and in Alzheimer's disease. *Nature* 1997;**389**:603–6.
36 **Thomas KA**. Vascular endothelial growth factor, a potent and selective angiogenic agent. *J Biol Chem* 1996;**27**:603–6.
37 **Koochekpour S**, Merzak A, Pilkington GJ. Vascular endothelial growth factor production is stimulated by gangliosides and TGF-β isoforms in human glioma cells in vitro. *Cancer Letts* 1996;**102**:209–15.
38 **Krupinski J**, Kumar P, Kumar S, *et al*. Increased expression of TGF-β1 in brain tissue after ischemic stroke in humans. *Stroke* 1996;**27**:852–7.
39 **Behzadian MA**, Wang X-L, Shabrawey M, *et al*. Effects of hypoxia on glial cell expression of angiogenesis-regulating factors VEGF and TGF-β. *Glia* 1998;**24**:216–25.
40 **Snowdon DA**, Greiner LH, Mortimer JA, *et al*. Brain infarction and the clinical expression of Alzheimer's disease. The nun study. *JAMA* 1997;**277**:813–17.
41 **Andreasen N**, Vanmechelen E, Van der Voorde A, *et al*. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow-up study. *J Neurol Neurosurg Psychiatry* 1998;**64**:298–305.
42 **Galasko D**, Chang L, Motter R, *et al*. High cerebrospinal fluid tau and low amyloid beta 42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. *Arch Neurol* 1998;**55**:937–45.
43 **Holmin S**, Mathiesen T. Intracerebral administration of interleukin-1-beta induces neuronal DNA-fragmentation, encephalitis and vasogenic edema. *J Neurosurgery* 2000;**92**:108–20.
44 **Sutton ET**, Thomas T, Bryant MW, *et al*. Amyloid-beta peptide induced inflammatory reaction is mediated by the cytokines tumor necrosis factor and interleukin-1. *J Submicrosc Cytol Pathol* 1999;**31**:313–23.